

Application No. 09/664,827
Amendment Dated May 18, 2004
Reply to Final Rejection of February 23, 2004

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Currently Amended) A multiplex structure comprising:

a first strand containing a first sequence of nucleobases;

a second strand containing a second sequence of nucleobases, wherein said second strand is associated with said first strand by Watson-Crick bonding;

a third strand containing a third sequence of nucleobases; and

a fourth strand containing a fourth sequence of nucleobases, wherein: (a) said fourth strand is associated with said second strand and said third strand by Watson-Crick bonding, (b) ~~and wherein~~ at least one nucleobase of said fourth sequence of nucleobases is associated by Watson-Crick bonding to at least one nucleobase of said third sequence of nucleobases and to at least one nucleobase of said second sequence of nucleobases, (c) each nucleobase in said second sequence and said fourth sequence binds to two other nucleobases, and (d) said multiplex structure is isolated, purified, artificial or synthetic.
2. (Canceled).
3. (Original) The multiplex structure of claim 1, wherein each said strand independently comprises a nucleic acid or a nucleic acid analogue.
4. (Original) The multiplex structure of claim 3, wherein each said strand

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independently comprises DNA or RNA.

5. (Original) The multiplex structure of claim 3, wherein each said strand independently comprises a nucleic acid analogue containing an uncharged or partially charged backbone.

6. (Original) The multiplex structure of claim 1, wherein one of said second strand or said fourth strand comprises DNA and the other of said second strand or said fourth strand comprises RNA, mRNA, hnRNA, rRNA, tRNA or cDNA.

7. (Original) The multiplex structure of claim 1, wherein said second strand and said fourth strand are anti-parallel to each other.

8. (Original) The multiplex structure of claim 7, wherein a major groove of said first strand and said second strand is placed in a major groove of said third strand and said fourth strand.

9. (Original) The multiplex structure of claim 1, wherein said second strand and said fourth strand are parallel to each other.

10. (Original) The multiplex structure of claim 9, wherein a major groove of said first strand and said second strand is placed in a minor groove of said third strand and said fourth strand.

11. (Canceled).

12. (Original) The multiplex structure of claim 1, wherein no strand is contiguous with another strand.

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13. (Original) The multiplex structure of claim 1, wherein said multiplex structure is substantially free of Hoogsteen bonding.

14. (Original) The multiplex structure of claim 1, wherein said multiplex structure is substantially free of G-G quartets.

15. (Original) The multiplex structure of claim 1, wherein said first strand and said second strand are 5 to 50 base pairs long.

16. (Original) The multiplex structure of claim 1, wherein said third strand and said fourth strand are genomic DNA.

17. (Original) The multiplex structure of claim 1, wherein said third strand and said fourth strand include a haplotype in genomic DNA.

18. (Original) The multiplex structure of claim 1, wherein said third strand and said fourth strand are PCR amplified products.

19. (Original) The multiplex structure of claim 1, wherein said multiplex structure is free of solid support.

20. (Currently Amended) ~~The A~~ multiplex structure of claim 1, ~~wherein comprising:~~
a first strand containing a first sequence of nucleobases;
a second strand containing a second sequence of nucleobases, wherein said second strand
is associated with said first strand by Watson-Crick bonding;
a third strand containing a third sequence of nucleobases; and

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a fourth strand containing a fourth sequence of nucleobases, wherein: (a) said fourth strand is associated with said second strand and said third strand by Watson-Crick bonding, (b) at least one nucleobase of said fourth sequence of nucleobases is associated by Watson-Crick bonding to at least one nucleobase of said third sequence of nucleobases and to at least one nucleobase of said second sequence of nucleobases, and (c) said multiplex structure is bound to a solid support.

21. (Currently Amended) The multiplex structure of claim 120, wherein said solid support is not electrically conductive.

22. (Currently Amended) The multiplex structure of claim 120, wherein said solid support is electrically conductive.

23. (Currently Amended) ~~The A~~ multiplex structure of claim 1, further comprising:

a first strand containing a first sequence of nucleobases;

a second strand containing a second sequence of nucleobases, wherein said second strand is associated with said first strand by Watson-Crick bonding;

a third strand containing a third sequence of nucleobases;

a fourth strand containing a fourth sequence of nucleobases, wherein said fourth strand is associated with said second strand and said third strand by Watson-Crick bonding, and wherein at least one nucleobase of said fourth sequence of nucleobases is associated by Watson-Crick bonding to at least one nucleobase of said third sequence of nucleobases and to at least one nucleobase of said second sequence of nucleobases; and

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a therapeutic, prophylactic or diagnostic agent bound to at least one of said first strand, said second strand, said third strand and said fourth strand.

24. (Currently Amended) ~~The A~~ A multiplex structure of claim 1, ~~wherein comprising:~~
a first strand containing a first sequence of nucleobases;
a second strand containing a second sequence of nucleobases, wherein said second strand
is associated with said first strand by Watson-Crick bonding;
a third strand containing a third sequence of nucleobases; and
a fourth strand containing a fourth sequence of nucleobases, wherein: (a) said fourth
strand is associated with said second strand and said third strand by Watson-Crick bonding, (b) at
least one nucleobase of said fourth sequence of nucleobases is associated by Watson-Crick
bonding to at least one nucleobase of said third sequence of nucleobases and to at least one
nucleobase of said second sequence of nucleobases, (c) said multiplex structure is isolated,
purified, artificial or synthetic, and (d) said first strand and said second strand are each 5 to 30
bases long and said third strand and said fourth strand are each 8 to 3.3 X 10⁹ base pairs long.

25. (Original) The multiplex structure of claim 1, wherein said fourth sequence contains 25% to 75% purine bases and 75% to 25% pyrimidine bases in any order.

26. (Withdrawn) A method for providing the multiplex structure of claim 1, said method comprising:

providing a hybridization medium comprising said first strand, said second strand, said third strand, said fourth strand, water, a buffer and at least one promoter; and

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incubating said hybridization medium for an incubation time effective to hybridize said second strand to said fourth strand to provide said multiplex structure.

27. (Withdrawn) The method of claim 26, wherein said hybridization medium is buffered to a pH of about 5 to about 9.

28. (Withdrawn) The method of claim 26, wherein said at least one promoter is an intercalating agent.

29. (Withdrawn) The method of claim 28, wherein said at least one promoter is an intercalating fluorophore, and a fluorescent intensity of a test medium containing said multiplex structure is directly correlated with a binding affinity of said second strand for said fourth strand.

30. (Withdrawn) The method of claim 29, wherein said intercalating fluorophore is a member selected from the group consisting of YOYO-1, TOTO-1, ethidium bromide, ethidium homodimer-1, ethidium homodimer-2 and acridine.

31. (Withdrawn) The method of claim 26, wherein said at least one promoter is tethered to at least one of said first strand, said second strand, said third strand and said fourth strand.

32. (Withdrawn) The method of claim 26, wherein said at least one promoter is a monovalent cation.

33. (Withdrawn) The method of claim 26, wherein said at least one promoter is a cation having a valency greater than one.

34. (Currently Amended) The method of claim ~~33~~26, wherein said at least one

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promoter is at least one cation ~~is at least one member~~ selected from the group consisting of alkali metal cations, alkaline earth metal cations, transition metal cations, $\text{Co}(\text{NH}_3)_6^{+3}$, trivalent spermidine and tetravalent spermine.

35. (Currently Amended) The method of claim 3332, wherein said cation is K^+ or Na^+ provided at a concentration of 50mM to 125mM.

36. (Withdrawn) The method of claim 26, wherein said third strand and said fourth strand are provided in said hybridization medium before said first strand and said second strand, and wherein said first strand and said second strand are provided in dehydrated form prior to rehydration by contact with said hybridization medium.

37. (Withdrawn) The method of claim 26, wherein said incubation time is not more than about two hours.

38. (Withdrawn) The method of claim 26, wherein said incubating is conducted at room temperature.

39. (Withdrawn) The method of claim 26, wherein hybridization of said second strand to said fourth strand is detected as a change in a fluorescent, chemiluminescent, electrochemiluminescent or electrical signal.

40. (Withdrawn) The method of claim 39, wherein an intensity of said signal is correlated with a binding affinity between said second strand and said fourth strand.

41. (Withdrawn) The method of claim 40, wherein at least one of said first strand and said second strand is covalently labeled with a non-intercalating fluorophore and said intensity is

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inversely correlated with said binding affinity.

42. (Withdrawn) The method of claim 41, wherein said non-intercalating fluorophore is a member selected from the group consisting of biotin, rhodamine and fluorescein.

43. (Withdrawn) The method of claim 41, wherein said method is a homogeneous assay conducted without providing a signal quenching agent on said target sequence or on said probe.

44. (Withdrawn) The method of claim 26, wherein hybridization of said second strand to said fourth strand inactivates an activity associated with at least one of said third strand and said fourth strand.

45. (Withdrawn) The method of claim 26, wherein at least one of said first strand and said second strand further comprises a pharmaceutical agent, and wherein hybridization of said second strand to said fourth strand places said pharmaceutical agent an effective distance from a target on said third strand, said fourth strand or on another molecule associated with at least one of said third strand and said fourth strand.

46. (Withdrawn) The method of claim 45, wherein said pharmaceutical agent is a member selected from the group consisting of nucleic acids designed to bind promoter sequences of clinically relevant genes, nucleic acids designed to bind clinically relevant genes, or nucleic acids designed to bind origin of replication sites of pathogens.

47. (Withdrawn) The method of claim 26, wherein a ratio of said first strand and said second strand to said third strand and said fourth strand is about 10:1.

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48. (Withdrawn) The method of claim 26, wherein concentrations of each of said first strand, said second strand, said third strand and said fourth strand are not more than 5×10^{-10} M.

49. (Withdrawn) The method of claim 26, wherein said at least one promoter is a minor groove nucleic acid binding molecule, which binds in a non-intercalating manner and binds with an association constant of at least 10^3 M^{-1} .

50. (Original) The multiplex structure of claim 1, wherein said first strand is associated with said third strand by Watson-Crick bonding.

51. (Original) An electrical circuit comprising the multiplex structure of claim 1.

52. (Canceled).